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Organic Management of *Meloidogyne incognita* (Root-knot Nematode) in *Dolichos lab lab* (Indian Bean) with Aqueous Extracts of *Pleurotus ostreatus* Oyster Mushroom Spent Compost

Chandrakala B [a*] and Y. Sunila Kumari [a]

[a] Department of Zoology, University College for Women, Osmania University, Hyderabad, Telangana 500095, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This investigation was done to determine the efficacy of oyster mushroom spent compost to control the root knot nematodes. The cultivation of oyster mushrooms yields spent mushroom compost (SMC), which has a unique microbiota that may include additional nematode antagonists and high levels of mycelium residual enzymes, and high humidity. These characteristics make SMC an excellent candidate for biological control. In order to reduce *Meloidogyne incognita* in Indian beans,

**Corresponding author: Email: machachandrakala@gmail.com;*

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this study evaluated the potential of an aqueous extract of SMC from *Pleurotus ostreatus* cultivation. Invitro experiments on second stage Juveniles (J2) larvae showed successful results with death of the larvae at 50% concentration.

Keywords: Meloidogyne incognita; Pleurotus ostreatus; Indian bean.

1. INTRODUCTION

"Root knot nematode belonging *Meloidogyne species* is a common root knot nematode infecting the majority of plants in India. There are quite a number of different species that infect the majority of crops, but among them M*. incognita, M. javanica, M. arenaria, and M. hapla* are considered as major ones causing huge destruction to the crop yield. It has been reported that *Meloidogyne,* species infect important vegetable crops like okra, tomato, French bean, pea, potato, capsicum, fodder maize, and mustard" (Sharma et al., 2018; Samiksha Jhamta and Neelam Thakur, 2023). "Nematicides are used to control root knot nematodes but are difficult for subsistence farmers on a small scale in developing countries. Since most nematicides are not only expensive but also harmful to human health and the environment. The addition of organic matter in the form of compost or manure will decrease nematode population and damage to crops" (Walker, 2004; Akhtar and Alam, 1993; Stirling, 1991). "This could be a result of improved soil structure and fertility, an increase of plant resistance, release of anti-nematode-toxins, or increased populations of fungal and bacterial parasites and other nematode-antagonistic agents" (Akhtar and Malik, 2000). There is a growing demand for usage of organic materials, to be used to control the root knot nematode more effectively. In this regard there are quite a number of alternative organic approaches have been in use as reported by various authors (Zakaria et al. (2013). Keeping in view the importance of root knot nematode management and to save the environment, the present study was conducted to assess the aqueous extract of spent compost of oyster mushrooms on larval mortality of Meloidogyne incognita collected from Indian bean.

2. MATERIALS AND METHODS

2.1 Collection of Root Galls

Galled roots of Indian bean were collected in plastic bags, appropriately labeled, and brought to the laboratory of the Department of Zoology, OUCW, Koti, Hyderabad. Telangana for further studies.

2.2 Identification of Nematode Species through Perineal Pattern Morphology

The extraction was done using the method described by Hussey and Barker (1973). Galled roots of plant were washed and galls cut open using a scalpel and a dissecting needle to tease out adult female nematodes in a petri dish containing water. Meloidogyne females' perineal patterns were cut using a method described by Taylor and Netsch (1974). "Cuticles of the female nematodes were ruptured by cutting and gently pushing out body tissues. Thirty samples of cuticles were then placed in 45% lactic acid in a petri dish, lactic acid aided in facilitating removal of body tissues and allowed to stand for half an hour. After tissues removal, the cuticle was transferred to a drop of glycerin where it was carefully trimmed so as to be only slightly larger than the perineal pattern. The piece of cuticle with the perineal pattern was transferred to a drop of glycerin on a slide". Observations were made on a compound microscope for identification as described by Taylor and Netsch (1974).

Fig. 1. Root galls collection

2.3 Perineal Pattern Diagram Confirmed the Identification of Female of *M. incognita*

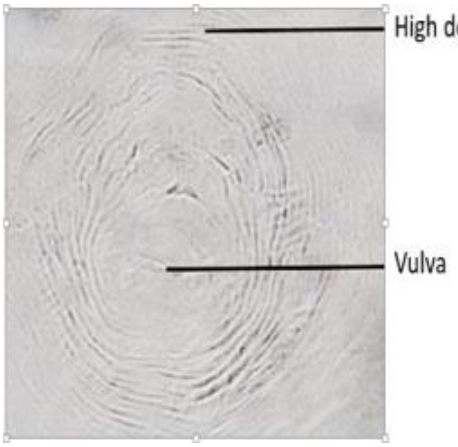

Fig. 2. Perineal pattern female of *M. incognita*

2.4 *Meloidogyne incognita* Juveniles (J2) Collection

2.4.1 Cobbs sieving method

The sieving technique is also known as the 'bucket-sieving' method. Although crude, it is widely used as it enables the extraction of large numbers of both active and inactive nematodes in a relatively short time. Equipment required includes two plastic buckets (5l), sieves of 15–20 cm diameter made with wire mesh (preferably stainless steel) of an aperture size of 2 mm, 710, 250, 125, 90, 63, 45 and 25 m, respectively and tall 100 ml beakers for the residue from the sieves. Usually only three or four of the set of sieves will be used for a particular sample, with the sieves selected to match the size of nematode it is hoped to extract, and to suit the type of soil involved. The method is as follows.

1. Mix the soil sample thoroughly and place a known volume of soil (100–500 ml) in bucket I and fill with about 1–4 l of water. Dry soils should be soaked for a few hours. The mixture is stirred to free nematodes from the soil and suspend them in the water. Flocculating agents, such as Separan NP10 (12.5 g/ml), might be used to help to break up soil aggregates in heavy clay soils.
2. Let the mixture settle for 30–60 s and decant over a 2 mm aperture sieve into bucket II. Avoid pouring the sediment. Add less water to the sediment in bucket I and repeat this step 2–3 times to increase nematode recovery. Any sediment left in bucket I is then discarded and bucket I washed out. The sieve is rinsed over bucket II. The residue on this sieve may contain very large nematodes, but usually it can safely be discarded. The contents of bucket II are stirred, allowed to settle for about 10 s and then poured through a 710 m aperture sieve into the clean bucket I, leaving behind heavy soil particles to which more water is added and the process repeated, if desired. The sieve over bucket I is rinsed. The residue on this sieve may contain only a few large nematodes, but this often depends on how much debris is present.
3. To collect the residue, hold the sieve over bucket I at a steep angle (35–45°) and direct a gentle stream of water on to its upper side to wash the nematodes to the bottom edge of the sieve. Small nematodes and eggs will be washed through the sieve into bucket I and recovered later. Transfer the nematodes on the sieve into a 250 ml beaker using a gentle stream of water, leaving behind any heavy particles.
4. Bucket II is cleaned and the process repeated using 250, 125 and 90 m aperture sieves and collecting the residues, as described above. The residues of each sieve can be pooled in one beaker or kept separate in different beakers. If the contents of the beakers appear cloudy, it is because the residue on the sieve was rinsed inadequately. If necessary, the contents should be poured back on to the sieve and rinsed again over the bucket containing the remaining

suspension before proceeding to the next sieve in the series. The contents of the collecting beakers are allowed to settle for 1–2 h and the supernatant liquid is carefully decanted or syphoned off leaving about 20 ml in the bottom. The material can be transferred to a viewing dish and examined.

2.5 Preparation of aqueous extract of Oyster Spent Mushroom Compost

SMC of oyster mushroom were collected from mushroom house of Krushi Defence Colony, Patancheru, Hyderabad. The SMC were sterilized using an autoclave at 121℃ for 15 min and stored in a dry condition. 500gms of SMS were taken and ground then the fine powder was soaked in distilled water at 1:1 and were stored for 24 hours at room temperature. After 24 hours, the extract was filtered through Whatman filter paper (20 µm).

2.6 Preparation of Serial Dilutions and Application

Serial dilutions i.e. 10%,20%,30%,40%and 50% (gm/100 ml) of the Oyster mushroom spent composts were prepared and Juvenile mortality bioassay were performed with the above filtrate. The data were recorded after 24 hrs and 48 hrs, 72 hrs and 96 hrs.

Fig. 3. Cobb decanting method for collection of small nematodes from the soil or endoparasitic nematodes from infected plant tissue

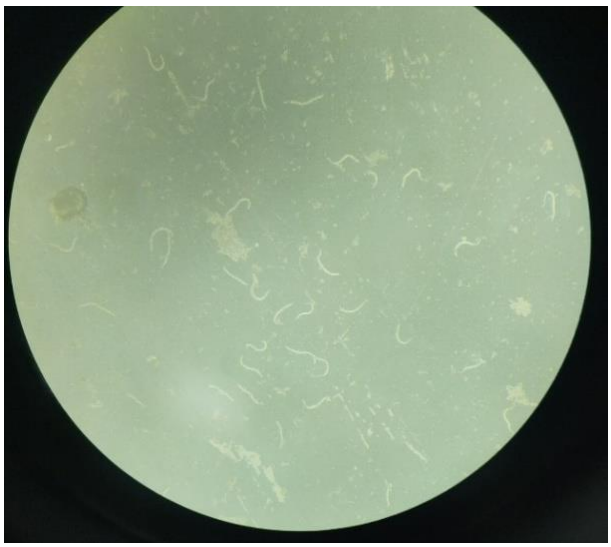

Fig. 4. *M. incognita* Juveniles observed under stereomicroscope

Fig. 5. Preparation of oyster spent mushroom compost

2.7 *In vitro* Analysis of Mushroom Spent Compost on *Meloidogyne*

To the 2 ml of the each SMC concentrations, 100 nematode juveniles were added and screened *in vitro* for their bio control efficiency against of *M. incognita* at different time periods. Velum prime served as chemical check**.** Control treatment contained only distilled water and all treatments were replicated four times. Treatments were left under ambient temperature of 25 ± 2^{o} C to determine the efficiency of MSC on nematodes. Tested materials were observed at 24hrs,48hrs, 72hrs and 96 hrs for juvenile mortality. Juveniles showing inactive straight posture or did not show any movement after prodding were considered dead. Mortality counts were observed using a research microscope under 10X magnification. The cumulative number of dead juveniles was calculated in comparison with the control treatment of distilled water.

2.8 *In vitro* Nematode (*M. incognita*) Mortality

The mortality percentages were calculated as the following equation:

Mortality (%) = (No.of dead juveniles / Total number of juveniles) x 100

Table 1. *In vitro* analysis of Mushroom Spent Compost on *M.juveniles* (J2) experiment Results

S.No.	Treatments (%) (gm/100 ml)	Nematode juvenile mortality (%)			
		24 hr	48 hr	72 hr	96 hr
1.	10%	2.8	4.6	6.6	8.25
2.	20%	6.6	8.4	11	14.75
3.	30%	38	42	47.6	56.00
4.	40%	47	73	77.2	91.00
5.	50%	52.8	74.4	80.2	94.50
6.	Control	0.2	0.4	0.6	0.75
7	Standard(velum prime)	40	68	70.2	83.25
	CD (P ≤0.001)	0.47	0.52	0.57	0.44
	SE (m)	0.16	0.18	0.19	0.15
	SE (d)	0.23	0.25	0.27	0.21
	C.V.	8.50	8.04	8.17	5.86

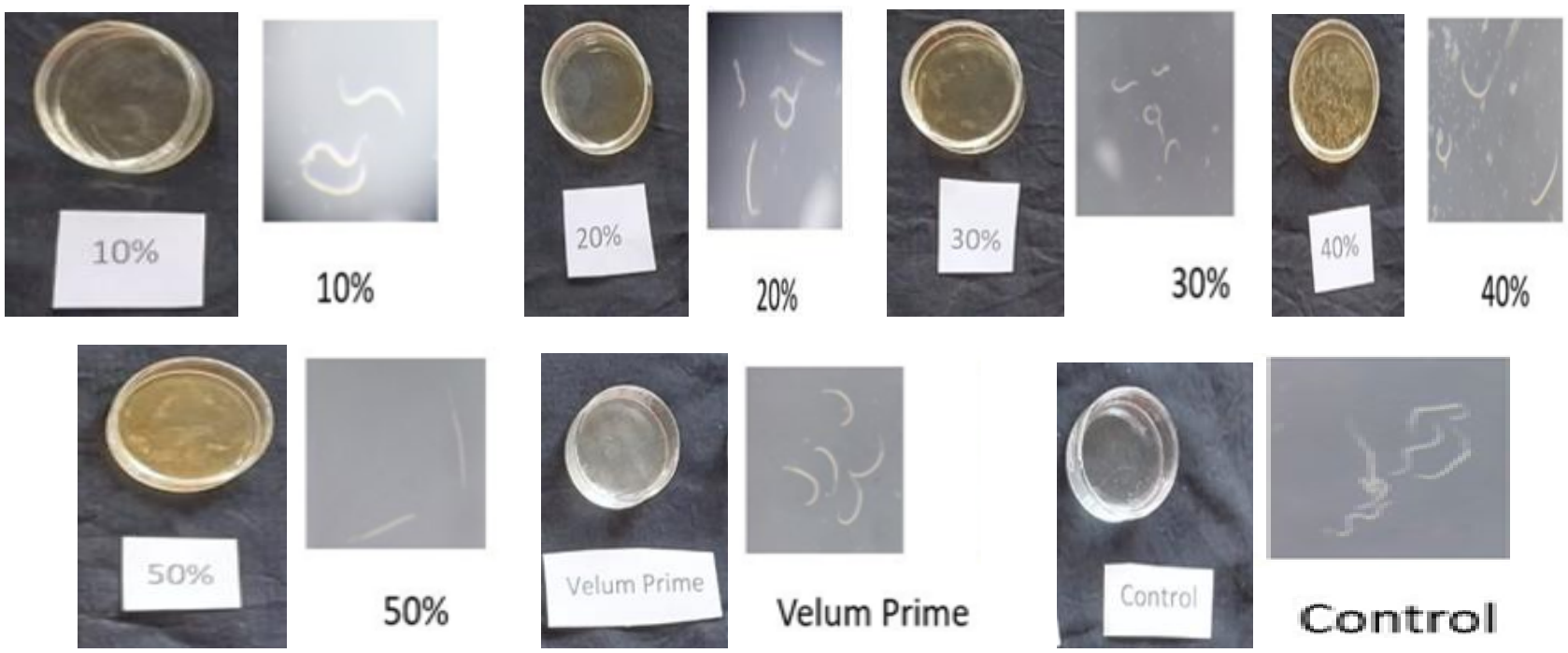

Plate 1. *In vitro* nematode mortality in Different concentration of Mushroom spent compost

3. RESULTS AND DISCUSSION

The present study was conducted to investigate *in vitro* activity of spent mushroom compost against the second stage juveniles of *Meloidogyne incognita* collected from Indian bean and it was found to be quite effective as a nematicide. *In vitro* study revealed that concentration 50% was more effective than other concentrations 40% and control treatment.

The present study, Oyster Mushroom Spent Compost, *Pleurotus ostreatus* was found to be quite effective in controlling the *Meloidogyne incognita* parasites of Indian bean i*n vitro.* Among the different dosages used, 50% concentration of Mushroom Spent Compost (MSC) was found to be very effective. These results coincide with studies of Zhong-Yan Yang *et.al,* (2023), who have reported the effective control of Plant Parasitic nematdes using mushroom spent compost. In their study, they had used the mushroom spent compost made using *Pleurotus* eryngii and studied its nematode suppressing activity and also the mechanisms underlying this suppressive effect. A similar kind of observation has been reported by Mai Nagah Abd Elmohsen Alhendy, (2021), who had observed the control root knot nematode infection in Eggplant, *Solanum Melongena* L. In this study, it was reported that there was significant reduction in the egg mass and also juvenile population of RKN when mushroom spent compost was used. In another study by Bakr, et al., (2022), also highlighted the use and effective control of root knot nematode using mushroom spent compost. In their study, the authors reported a significant decline in gall formation and also egg masses on tomato plants. They opined that the nematicidal effect of MSC may be because of phenolic compounds released by the compost in to the soil. Similarly authors like Mian and Rodriguez-Kabana, 1982) suggested that the nematicidal effect of MSC may be attributed to phenols, terpenes and tannins, which get released in to the soil by the compost or may also be due to derived decomposition products such as ammonia, nitrites and hydrogen sulphides. (Rodriguez-Kabana, 1986). Studies by Raman Kumar et al., (2020) illustrated the use of different kinds of nematicidal fungal species, to control different *myceliophagous n*ematodes. In their study, they used different fungal species, to control different myceliophagous nematodes such as *Aphelenchus spp., Aphelenchoides spp. and Ditylenchus myceliophagus*. In their study, they concluded that certain fungal species such as *Arthrobotrys oligospora,* have the capacity to trap the nematodes and prey upon them, thus controlling their populations. Therefore based upon these studies, it may be said that, the present nematicidal activity observed *In vitro* using MSC on *Meloidogyne incognita* may be attributed to either release of certain phenolic or tannin compounds by the compost or may also be due to the nematode trapping capability of oyster mushroom, *Pleurotus ostreatus* compost which may be further explored in future.

In summary it may be said that the oyster mushroom compost which was prepared and used in different concentrations seems to have a significant nematicidal activity upon *Meloidogyne incognita* which frequently infects, the Indian bean, may be attributed to its phytochemical constituents.

4. CONCLUSION AND RECOMMENDA-TIONS

Aqueous extracts of oyster mushroom composts were found effective for the organic management of root-knot nematodes. The application of oyster compost was found effective on root-knot nematode juveniles' mortality.

Oyster Spent mushroom compost can be used as an effective tool to manage root-knot nematodes and save the environment from the effect of nematodes. Further research work is needed to investigate the effect of spent mushroom compost on the management of root knot nematodes and other soil borne diseases. How spent mushroom compost can help in improving the soil health, also needs an intensive research.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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